Carbohydrate and Peptide-Conjugated Surfaces For High Throughput Screening Of Candidate Tissue Engineerirng Scaffold Materials

Catherine M.Klapperich,[1], Jie Song,[1], Carolyn R.Bertozzi[1,2,3,4]

[1] Lawrence Berkeley National Laboratory, Materials Sciences Division, 1 Cyclotron Dr., Berkeley, CA 94720, USA; [2] University of California, Department of Chemistry, Berkeley, CA 94720, USA; [3]Department of Molecular and Cell Biology, Berkeley, CA 94720, USA; [4]Howard Hughes Medical Institute, Berkeley, CA 94720 USA. bertozzi@cchem.berkeley.edu

Abstract Summary: In order to isolate cell/biopolymer interactions, a model system is needed in which cells interact with surface molecules or gradients of surface molecules in the absence of confounding factors like topography and pore size. We present a test system that allows higher throughput screening of potential scaffold materials to precede more time-consuming fabrication of 3-dimensional materials.

Background: Many cell adhesion events involve glycosylated proteins. Some chemokines are known to bind to glycosaminoglycans in the extracellular matrix. By binding to components of the extracellular matrix and proteoglycans on cell surfaces, these chemicals can form a gradient that will attract the target cells to a more well defined location[1]. Similar processes are active in cell growth and tissue regeneration[2].

These cell adhesion events are important to the design of novel biomaterials where carbohydrates and peptides are used to decorate artificial surfaces and three-dimensional scaffolds to enhance their bioactivity and biocompatibility. In this work, we made a series of synthetic surfaces that can display a range of carbohydrates and peptides. By exposing cells to these surface groups in a controlled environment and tracking how the cells respond, we will start to learn what to expect when cells are incorporated into a three-dimensional tissue-engineering scaffold containing similar chemical handles.

Experimental Methods: To make carbohydrate-terminated surfaces, we used amino terminated slides obtained from 4th State, Inc. (Belmont, CA). These slides present NH_2 groups at the surface and were used to couple with the carboxylates displayed on the scaffold of chondroitin-6-sulfate (c6s). To make the peptide conjugated slides, we treated a subset of the NH_2-terminated slides with 25% glutaraldehyde for 24 hours to generate aldehyde-terminated slides. We then functionalised these slides with a number of peptides via Schiff base formation.

We conjugated c6s to the NH_2-terminated slides using N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry. We submerged the slides at room temperature in a 60ml buffer solution of 0.1M 2-morpholinoethanesulfonic acid sodium salt (MES), 0.5M NaCl and 0.45mM hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (sulfo-NHS), followed by the addition of c6s (50mg) and 100µl of 76mM aqueous EDC solution. The reaction was allowed to proceed at room temperature for 24 hours over an orbital shaker.

The aldehyde-terminated slides were submerged in an aqueous solution of 22 nM peptide at pH 7. The free N-terminus of the peptide was coupled with the surface aldehydes via the formation of Schiff base at room temperature. After 24 hours, the functionalised slides were washed in flowing Millipore water for 15 minutes and air dried in a biohood. They were stored in a desiccator until further surface analysis.

Results and Discussion:

XPS (ESCA) Analysis of Surface Groups: X-ray photoelectron spectroscopy was used to confirm the covalent coupling of our peptides and carbohydrates to the glass surfaces. The test peptide RGDC and c6s both contain sulfur atoms, which are not present in scans of the unreacted NH_2-terminated slides. Using elemental analysis, we were able to demonstrate the presence of S atoms on the glass surfaces reacted with RGDC and c6s as the terminal conjugates.

SIMS Analysis: Secondary ion mass spectrometry was used as a complement to the XPS results. We wanted to confirm that the S atoms detected in XPS were from the conjugated polymers and not the co-reactant sulfo-NHS. Preliminary SIMS data shows some S containing fragments on the control slides. We need to investigate this result further.

Contact Angle Measurements: We characterized the surface energy of the functionalised surfaces using water contact angle measurements. A lower contact angle corresponds to a more hydrophilic material surface. As expected, the contact angles of the peptide and carbohydrate conjugated slides are lower than both the control slide and the glutaraldehyde treated slides.

Slide Sample	Advancing	Receding
NH_2 terminated (unreacted)	60	59
NH_2 – glutaraldehyde	47	51
NH_2 – chondroitin sulfate	41	39
NH_2 – RGDC	34	38

Preliminary Cell Adhesion Studies: In order to demonstrate that the surfaces derived from our coupling technique were not intrinsically toxic to cells, we exposed several of the treated slides to IMR-90 human fibroblast cells. Qualitative observation showed that all of the surfaces allowed for fibroblast adhesion and spreading.

Conclusion: We have demonstrated in this work the fabrication of a simple and flexible surface chemical system tethered with carbohydrates and peptides for the investigation of cellular adhesion events involved in tissue regeneration and repair.

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